genomic DNA with P1 (150 nM) and P2 (300 nM) as primers for the PCR [K. B. Mullis and F. A. Faloona, *Methods Enzymol.* **155**, 335 (1987)]. P1 is an oligomer whose sequence is shifted 1 nt in the 5′ direction with respect to the JH-27 oligomer to place the two changes from consensus at its 3′ end; P2 is a single-copy oligomer located at 17 to 36 bp 3′ to L1.2A. The 1836-bp PCR fragment was sequenced with the use of an internal primer [R. K. Saiki *et al.*, *Science* **239**, 487 (1988); C. Wong *et al.*, *Nature* **330**, 384 (1987)].
22. Genomic DNA (100 μg) from the mother of patient JH-27 was partially digested with Bam HI (10 μg of DNA per digest). Digests were pooled and centrifuged in a 10 to 40% sucrose gradient at 60,000*g* for 22 hours. Aliquots containing fragments of 10 to 18 kb were pooled, dialyzed, ligated to digested EMBL3 bacteriophage arms, and packaged (Stratagene). Recombinants (2.1×10^6) were screened with the three L1 probes (Fig. 2), and one recombinant containing L1.2B was purified, and phage DNA was isolated.
23. The L1.2B element was amplified by PCR, and we sequenced overlapping 1-kb products of both strands on a DNA sequencer (Applied Biosystems) using cycle sequencing with Taq polymerase and dye-labeled dideoxynucleotide terminators.
24. M. L. Budarf, M. Simon, B. S. Emanuel, *Cytogenet. Cell Genet.* (abstr.), in press.
25. R. Holmquist, M. M. Miyamoto, M. Goodman, *Mol. Biol. Evol.* **5**, 217 (1988).
26. M. Goodman *et al.*, *Genome* **31**, 316 (1989); R. J. Britten, *Science* **231**, 1393 (1986).
27. R. J. Britten, D. B. Stout, E. H. Davidson, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3718 (1989).
28. G. D. Swergold, *Mol. Cell. Biol.* **10**, 6718 (1990); A. V. Furano, S. M. Robb, F. T. Robb, *Nucleic Acids Res.* **16**, 9215 (1988).
29. L. J. Mizrokhi *et al.*, *Cell* **54**, 685 (1988).
30. J. Skowronski, M. F. Singer, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 457 (1986).
31. GenBank accession numbers are as follows: L1.1, M80340; L1.1 3′ flanking sequence, M80341; L1.1 5′ flanking sequence, M80342; L1.2 alleles, M80343; L1.2 3′ flanking sequence, M80344; and L1.2 5′ flanking sequence, M80345.
32. We thank M. Singer, G. Swergold, T. Fanning, R. Thayer, L. Shapiro, and S. Holmes for helpful discussions, and patient JH-27 and his parents for their contribution to this work. Supported in part by NIH grants (to H.H.K. and A.F.S.). B.A.D. is supported by a fellowship from the Sloan Foundation. S.L.M. is supported by an NIH predoctoral training grant in human genetics.

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Reverse Transcriptase Encoded by a Human Transposable Element

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L1 elements are highly repeated mammalian DNA sequences whose structure suggests dispersal by retrotransposition. A consensus L1 element encodes a protein with sequence similarity to known reverse transcriptases. The second open reading frame from the human L1 element L1.2A was expressed as a fusion protein targeted to Ty1 virus-like particles in *Saccharomyces cerevisiae* and shown to have reverse transcriptase activity. This activity was eliminated by a missense mutation in the highly conserved amino acid motif Y/F-X-D-D. Thus, L1 represents a potential source of the reverse transcriptase activity necessary for dispersion of the many classes of mammalian retroelements.

LONG INTERSPERSED ELEMENTS (LINE-1 or L1) are a large class of mammalian repeated sequences with structural similarities to retrotransposons (*1*). The predicted protein encoded by the second open reading frame (ORF2) of L1 elements contains domains similar to retroviral and other reverse transcriptases (RT) (*2, 3*). However, the classification of a sequence as a retrotransposon based solely on structural similarities or the presence of RT amino acid sequence motifs is tenuous; RT sequence similarity does not by itself prove RT activity. The availability of L1.2A, a putatively functional human L1 element (*4*), presented an opportunity to test directly for RT activity.

To assay the ORF2 protein encoded by L1.2A for RT activity, we used an expression system (*5*) based on retrotransposon Ty1 of *Saccharomyces cerevisiae* (*6*) (Fig. 1). Ty1 encodes two overlapping ORFs. The first, *TYA*, encodes a *gag*-like protein. The second, *TYB*, encodes a *pol*-like protein that is expressed as a fusion protein with TYA protein. Ty1 proteins and RNA are coassembled into virus-like particles (VLPs). We used a Ty1-L1 chimeric construct (pSM2) in which the L1.2A ORF2 was fused in-frame with *TYB* immediately 3′ to the protease domain, replacing the integrase, RT, and RNAseH domains of *TYB* (Fig. 1). As a negative control, the sequence between the fusion site and the downstream long terminal repeat (LTR) was deleted (pSM5). The parent plasmid, which contained a functional Ty1 element (pSM1), was used as a positive control. All constructs were regulated by the *GAL1* promoter. Upon galactose induction, yeast carrying these plasmids produced and accumulated large amounts of VLPs, which were then partially purified and assayed for RT activity. To reduce RT activity attributable to expression of endogenous Ty1 and Ty2 elements, an *spt3* host strain was used. Transcripts initiated from the intact endogenous Ty1 LTR promoter are reduced 10- to 20-fold in *spt3* strains, whereas transcription of *GAL1*-promoted elements is unaffected (*5, 7–9*).

Yeast cell extracts containing VLPs were fractionated on sucrose gradients and the fractions were assayed for RT activity (Fig. 2A). The L1-associated activity sedimented with a slightly slower velocity than the Ty1 RT. This may be due to an altered structure of the L1-Ty1 VLPs. No RT activity was detected in VLPs from the negative control plasmid, pSM5. The same fractions used for the RT profiles were immunoblotted with an antiserum raised against Ty1-VLPs (*10*) (Fig. 2B). The L1 RT peak cofractionated with TYA proteins, as did the Ty1 RT. VLPs were produced from the negative control construct (pSM5) (Fig. 2B), therefore absence of RT from pSM5 was not due to a lack of VLP production.

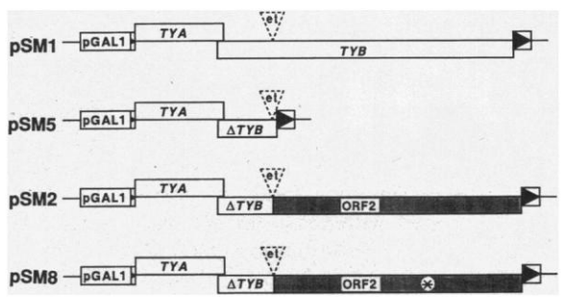

Fig. 1. Summary of plasmid inserts. Plasmids were constructed as described (*22*) and carry the yeast 2 μm plasmid origin of replication, the yeast selectable genes *URA3* and *TRP1*, and the bacterial selectable gene *bla*. pGAL1, *GAL1* promoter; *TYA*, first open reading frame of Ty1; *TYB*, second open reading frame of Ty1; Δ*TYB*, *TYB* truncated at base pair 2173; black triangles, Ty1 LTRs; ORF2, second open reading frame from L1.2A (*4*); dashed triangles, epitope tags (et) inserted in the parent plasmids to create pSM1et, pSM5et, pSM2et, and pSM8et (*16*). The asterisk indicates a missense mutation which produces a D to Y substitution in the RT domain of ORF2 (*22*).

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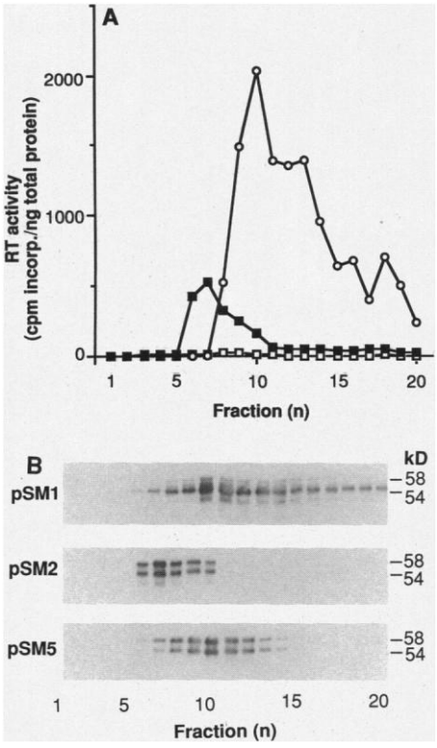

Fig. 2. RT activity co-fractionates with TYA proteins in VLPs. Yeast strains carrying the test plasmids (Fig. 1) were induced with galactose and VLPs were isolated (*23*). (**A**) Aliquots (2.5 μl) of individual sucrose gradient fractions were assayed for RT activity (*12*). pSM1, open circles; pSM2, solid squares; pSM5, open squares. Fraction 1 corresponds to the top of the gradients. (**B**) Aliquots (5 μl, pSM1 and pSM5; or 10 μl, pSM2) of the same fractions were separated by SDS-polyacrylamide gel electrophoresis, and after electrophoresis, the proteins were transferred to nitrocellulose. TYA proteins were detected by immunoblotting with antiserum raised against Ty1 VLPs (*10*) (1:1000 dilution), secondarily incubated with iodinated protein A, and visualized by autoradiography. TYA is present in two forms: a 58-kD precursor and a 54-kD processed form (*24*). Larger volumes of pSM2 fractions were necessary in order to obtain similarly exposed blots.

Table 1. L1 and Ty1 RTs: Substrates and micrococcal nuclease pretreatment.

Template	Primer	L1 RT*		Ty1 RT*	
		Untreated	MNase†	Untreated	MNase†
poly(rC)	oligo(dG)	118 (100)	756 (100)	1268 (100)	1872 (100)
–	oligo(dG)	2.0 (1.7)	4.4 (0.58)	2.0 (0.16)	9.6 (0.51)
poly(rC)	–	19.2 (16)	96 (12)	0.8 (0.06)	4.8 (0.26)
–	–	1.6 (1.4)	0.32 (0.04)	0.8 (0.06)	2.0 (0.11)
poly(rA)	oligo(dT)	69.6 (100)	484 (100)	760 (100)	1248 (100)
–	oligo(dT)	3.2 (4.6)	0.4 (0.08)	149 (20)	9.6 (0.77)
poly(rA)	–	4.4 (6.3)	0.8 (0.16)	1.6 (0.21)	1.2 (0.10)

*fmoles dNTP incorporated, calculated as described (*5*). RT assays were performed as described (*12, 13*). Numbers in parentheses are percentages of activity relative to reactions containing both primer and template. †VLPs were incubated in 100 μg per ml micrococcal nuclease (Boehringer Mannheim) and 7.5 mM $CaCl_2$ for 15 minutes at room temperature. Reactions were stopped by adding 0.33 volumes of 30 mM EGTA. VLPs were subsequently assayed as described (*12, 13*). (–) represents substrate not added.

The properties of L1 RT were determined with the use of pooled peak fractions (Table 1). With poly(rA) and oligo(dT) as template and primer, respectively, the L1 RT exhibited an absolute requirement for exogenous template and primer. When poly(rC) and oligo(dG) were used, template dependence was still observed, but approximately 16% of the activity was primer-independent. To determine whether this was due to endogenous nucleic acids in the VLPs acting as primers, VLPs were pretreated with micrococcal nuclease. Ca^{2+} ions were then chelated to inactivate the nuclease, and the VLPs were assayed for RT activity (Table 1). Micrococcal nuclease pretreatment reduced primer-independent activity to 6 to 12% of control. We have observed that oligo(dG) is a poorer substrate for micrococcal nuclease than oligo(dT) (*11*). The residual primer-independent activity observed with poly(rC) and oligo(dG) may therefore result from endogenous dG-rich primers.

The stimulation of RT activity by nuclease pretreatment was unexpected. As with the RT from the LINE-like *Crithidia* retrotransposable element 1 (CRE1) (*5*), this effect was much greater for L1 RT (6- to 7-fold) than for Ty1 RT (1.5-fold). Endogenous nucleic acids within the VLPs may inhibit the exogenous RT reaction by competing with exogenous primer and template for enzyme. The greater stimulation observed for L1 RT compared to Ty1 RT may reflect a higher affinity of L1 RT for endogenous nucleic acids, or there could be a higher concentration of nucleic acids in Ty1-L1 VLPs than in Ty1-VLPs.

As further characterized (Table 2), L1 RT activity required a divalent cation, preferring Mg^{2+} to Mn^{2+}. L1 RT maintained activity over a broad pH range and was slightly inhibited by 50 mM NaCl. Like other RTs, L1 RT could use several primer-template combinations, including DNA templates.

L1 RT and Ty1 RT differ in their affinity for 2′,3′-dideoxyNTPs (ddNTPs). With our standard assay conditions (*12, 13*), incorporation of dTTP by Ty1 RT was inhibited by 25% in the presence of 200 nM ddTTP, whereas incorporation by L1 RT was inhibited by 35% in the presence of only 0.2 nM ddTTP. The L1 RT products from these reactions were visualized by polyacrylamide gel electrophoresis and autoradiography, and appeared as a ladder extending to more than 600 nucleotides in length. The products decreased in size and quantity as the ddTTP concentration increased. Similar results were obtained using 2′,3′-dideoxyGTP with poly(rC) and oligo(dG) (*11*).

All known and putative RTs contain a pair of adjacent aspartic acid residues within a Y/F-X-D_1-D_2 amino acid motif (*3, 14*). A previous study of recombinant HIV RT showed that replacing the second D residue (D_2) eliminated RT activity (*15*). Therefore, we constructed the plasmid pSM8 (Fig. 1) with a mutant ORF2 in which the D_2 residue was changed to Y. RT activity derived from pSM8 was 40-

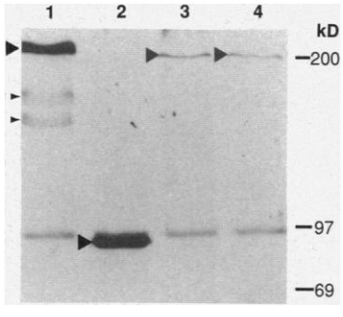

Fig. 3. Immunoblot of epitope-tagged VLPs. Full-length fusion proteins are indicated by the large arrowheads. VLP preparations (100 μg protein per lane) from yeast containing epitope-tagged plasmids pSM1et (lane 1), pSM5et (lane 2), pSM2et (lane 3), and pSM8et (lane 4) were separated by SDS–polyacrylamide gel electrophoresis and transferred to nitrocellulose. Proteins containing the epitope tag were detected by immunoblotting with a 1:7500 dilution of ascites fluid containing the monoclonal antibody 12CA5 (*16, 17*) (BAbCo). Immunoreactive bands were visualized with sheep antiserum to mouse IgG conjugated to horseradish peroxidase and enhanced chemiluminescence (ECL) reagents (both from Amersham) according to the manufacturer's protocols. The two bands indicated by small arrowheads in Ty1-VLPs from pSM1et are likely intermediates in TYB processing (*25*). No such intermediates are seen associated with VLPs derived from plasmids pSM5et, pSM2et, or pSM8et; these proteins are apparently not cleaved by TYB protease under these conditions. The protein migrating at 95 kD, visible in all four lanes, is likely a cross-reacting protein in the yeast extracts, as it is not present if the VLPs are further purified over a linear sucrose gradient (*11*).

Table 2. Properties of L1 RT.

Reaction condition	Activity
poly(rC):oligo(dG)	357 (100)*
poly(rA):oligo(dT)	246 (68.9)*
poly(rG):oligo(dC)	54 (15.1)*
poly(dC):oligo(dG)	291 (81.5)*
minus $MgCl_2$	1.05 (0.42)†
minus $MgCl_2$ + 10 mM $MnCl_2$	12.0 (4.7)†
pH 7.0	224 (89)†
pH 9.0	262 (104)†
+ 50 mM NaCl	200 (79)†
+ 50 mM KCl	254 (101)†

*Fentomoles dNTP incorporated after micrococcal nuclease pretreatment (*5, 12*). Numbers in parentheses are percentages of activity relative to the poly(rC):oligo(dG) reaction. †Fentomoles dGTP incorporated after micrococcal nuclease pretreatment (*5, 12*). Numbers in parentheses are percentages of activity relative to that obtained with the standard reaction mix (*12*) (252 fmoles for this experiment).

fold (with poly-rA and oligo-dT as substrates) or 90-fold (with poly-rC and oligo-dG) less than wild-type, and was equivalent to that derived from pSM5, which lacks ORF2 (*12*).

To visualize wild-type and mutant fusion proteins, we constructed versions of the test plasmids (Fig. 1) tagged with a synthetic DNA sequence encoding an epitope (*16*) that is recognized by a monoclonal antibody (*17*). Immunoblots, performed on epitope-tagged VLPs, detected full-length fusion proteins of the predicted sizes (Fig. 3), indicating that all fusion proteins were stable. In an immunoblot of serial twofold dilutions of epitope-tagged wild-type (pSM2et) and mutant (pSM8et) VLPs, the fusion proteins were not visible in either sample below a fourfold dilution. Therefore, there was at most a twofold difference in the amount of fusion proteins in these two samples. In all cases the epitope-tagged VLPs had amounts of RT activity comparable to the untagged versions (*11*).

We show here that ORF2 from a human L1 element encodes an RT activity, functionally corroborating previously defined sequence similarities between L1 and known retrotransposable elements. Evidence indicates that LINE-like elements encode RT (*5, 18*) and transpose through cytoplasmic RNA intermediates (*19*). LINEs, processed pseudogenes, and *Alu* repeats are all interspersed sequences with characteristics suggesting that they are derived from integrated reverse transcripts (*20*). These retroelements are potential mediators of a variety of genomic events such as gene duplication, deletion, and insertional mutagenesis (*21*). As a potential source of the RT responsible for the dispersion of these sequences, L1 may have contributed to the evolution of mammalian genomes.

REFERENCES AND NOTES

1. C. A. Hutchison III, S. C. Hardies, D. D. Loeb, W. R. Shehee, M. H. Edgell, in *Mobile DNA*, D. E. Berg and M. M. Howe, Eds. (American Society for Microbiology, Washington, DC, 1989) pp. 593–617; T. G. Fanning and M. F. Singer, *Biochim. Biophys. Acta* **910**, 203 (1987); A. F. Scott *et al.*, *Genomics* **1**, 113 (1987).
2. M. Hattori, S. Kuhara, O. Takenaka, Y. Sakaki, *Nature* **321**, 625 (1986); D. D. Loeb *et al.*, *Mol. Cell. Biol.* **6**, 168 (1986).
3. Y. Xiong and T. H. Eickbush, *EMBO J.* **9**, 3353 (1990).
4. B. A. Dombroski, S. L. Mathias, E. Nanthakumar, A. F. Scott, H. H. Kazazian Jr., *Science* **254**, 1805 (1991).
5. A. Gabriel and J. D. Boeke, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9794 (1991).
6. J. D. Boeke, in *Mobile DNA*, D. E. Berg and M. M. Howe, Eds. (American Society for Microbiology, Washington, DC, 1989), pp. 335–74.
7. F. Winston, K. J. Durbin, G. R. Fink, *Cell* **39**, 675 (1984).
8. J. D. Boeke, C. A. Styles, G. R. Fink, *Mol. Cell. Biol.* **6**, 3575 (1986).
9. All experiments were done in yeast strain AGY9, created by introducing the *spt3-101* mutation into strain YH82 (*MATa ura3-52 trp1Δ63 leu2Δ1 his4-539 lys2-801*) [H. Xu and J. D. Boeke, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8360 (1990)] with the use of the method of Winston *et al.* (7). Transformants were selected on SC-ura medium. All media were prepared as described [F. Sherman, G. R. Fink, C. W. Lawrence, *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1978)].
10. H. Xu and J. D. Boeke, *Mol. Cell. Biol.* **11**, 2736 (1991).
11. S. L. Mathias and A. Gabriel, unpublished results.
12. RT assay conditions used were modified from previously described conditions (*5, 13*). The standard reaction mix contained final concentrations of 10 μg/ml poly(rC), 0.7 mg/μl oligo$(dG)_{12-18}$, 180 nM dGTP (all from Pharmacia), 10 mM $MgCl_2$, 50 mM tris-HCl pH 8.0, 2% β-mercaptoethanol, and 60 μCi/ml α[^{32}P]dGTP (NEN). Alternate primer, template, and nucleoside triphosphates were used at the same concentrations. VLPs were incubated in the above mixture for 60 minutes at 30°C. Incorporation of radiolabeled nucleoside triphosphates into high molecular weight homopolymeric DNA was determined by scintillation counting of aliquots of the reactions that had been spotted onto diethylaminoethyl cellulose filters (DE81, Whatman) and washed as described [S. Goff, P. Traktman, D. Baltimore, *J. Virol.* **38**, 239 (1981)].
13. D. J. Eichinger and J. D. Boeke, *Cell* **54**, 955 (1988).
14. Abbreviations for the amino acid residues are: A, Ala; D, Asp; F, Phe; L, Leu; P, Pro; S, Ser; V, Val; Y, Tyr; X, variable.
15. B. A. Larder, D. J. M. Purifoy, K. L. Powell, G. Darby, *Nature* **327**, 716 (1987).
16. P. A. Kolodziej and R. A. Young, *Methods Enzymol.* **194**, 508 (1991); I. A. Wilson *et al.*, *Cell* **37**, 767 (1984). Epitope-tagged plasmids were constructed by inserting the double-stranded oligonucleotide pair 5′TCGACTACCCATACGATGTTCCAGATTACGCTAGCC3′, 3′GATGGGTATGGTACAAGGACTAATGCGATCGGAGCT5′ at the unique *Sal*I site in each of the plasmids pSM1, pSM2, pSM5, and pSM8. This oligonucleotide pair encodes the epitope (YPYDVPDVASL) from hemagglutinin of the influenza virus (HA1).
17. J. Field *et al.*, *Mol. Cell. Biol.* **8**, 2159 (1988); H. L. Niman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4949 (1983).
18. V. A. Ivanov, A. A. Melnikov, A. V. Siunov, I. I. Fodor, Y. V. Ilyin, *EMBO J.* **10**, 2489 (1991).
19. A. Pelisson, D. J. Finnegan, A. Bucheton, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4907 (1991); S. Jensen and T. Heidmann, *EMBO J.* **10**, 1927 (1991); J. A. Kinsey, *Genetics* **126**, 317 (1990); S. L. Martin, *Mol. Cell. Biol.* **11**, 4804 (1991); J. A. Kinsey, personal communication.
20. A. M. Weiner, P. L. Deininger, A. Efstratiadis, *Annu. Rev. Biochem.* **55**, 631 (1986).
21. H. M. Temin, *Mol. Biol. Evol.* **2**, 455 (1985); D. Baltimore, *Cell* **40**, 481 (1985); H. H. Kazazian, Jr., *et al.*, *Nature* **332**, 164 (1988); M. R. Wallace *et al.*, *ibid.* **353**, 864 (1991); S. Shen, J. L. Slightom, O. Smithies, *Cell* **26**, 191 (1981).
22. The parent plasmid (pSM1) was constructed as follows. The 4254-bp *Bst* EII-*Bam* HI fragment (including from the protease domain through downstream of the 3′ LTR) from pJEF1114 [G. Natsoulis, W. Thomas, M. C. Roghmann, F. Winston, J. D. Boeke, *Genetics* **123**, 269 (1989)] was replaced by the corresponding *Bst* EII-*Bam* HI fragment of pJEF1267 [E. Jacobs, M. Dewerchin, J. D. Boeke, *Gene* **67**, 259 (1988)]. Next, the *URA3* gene [M. Rose, P. Grisafi, D. Botstein, *ibid.* **29**, 113 (1984)] was inserted at the unique *Asp* I site by blunt-end ligation after all overhangs had been filled in with the large fragment of *E. coli* DNA polymerase I. The plasmid pSM5 was constructed by digestion of pSM1 with *Sal* I and *Sac* I, the overhangs were blunted, and the large fragment was religated intramolecularly. The ORF2-coding fragment used for the construction of pSM2 was derived as follows. Plasmid pL1.2A, a subclone of λL1.2A (*4*), was digested with *Sal* I and *Bgl* II, and the sequence from the *Sal* I site (5′ polylinker) to the *Bgl* II site (2170 of L1.2A) was replaced by a PCR product generated with the primers SM8 (5′AGCTAA CGTCGACATGACAGGATCA3′) and L1-2347 (5′ACTAGGATTGCAACCCTGC3′) and digested with both Sal I and Bgl II. Primer SM8 generates an in-frame *Sal* I site immediately 5′ to the first ATG codon of ORF2. The 4079-bp *Sal* I to *Sac* I (3′ polylinker) fragment containing ORF2 was then ligated to the large *Sal* I to *Sac* I fragment of pSM1, resulting in the plasmid pSM2. Plasmid pSM8 was produced by site-directed mutagenesis [T. A. Kunkel, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 488 (1985)] of pSM2 using the oligonucleotide SM11 (5′AATCATGTCATATGCAAACAG3′) whose sequence corresponds to the complement of nucleotides 4084 to 4105 of L1.2A; mismatches generate a new *Nde*I site and produce a missense mutation resulting in a D to Y substitution at amino acid 701 of ORF2.
23. VLPs were isolated as described (*5, 13*), except that cells were grown as follows: Cultures (100 ml) of yeast cells (starting at an OD_{600} of 0.5 to 1.0) transformed with the plasmids of interest were grown at 30°C for 24 hours in Yeast Nitrogen Base (YNB)-casamino acids medium [5 g per l $(NH_4)_2SO_4$, 2% (w/v) casamino acids, 1.7 g/l YNB] containing 0.1% glucose. The cultures were then diluted 1:5 into YNB-casamino acids medium containing 2% galactose and grown at 22°C for 24 hours. Linear gradient fractions were treated in one of two ways. Either individual fractions were centrifuged for 30 minutes at 65,000 rpm in a Beckman TLA-100 rotor and resuspended in 0.2 volumes of buffer B/EDTA (Fig. 2), or peak fractions from the linear gradient (step gradient in Fig. 3) were pooled and VLPs pelleted by centrifugation for one hour at 35,000 rpm in a Beckman SW41 rotor (all other experiments). These pooled VLPs were resuspended in 250 μl of buffer B/EDTA, aliquoted and stored at −70°C.
24. F. Müller, K. Brühl, K. Freidel, K. V. Kowallik, M. Ciriacy, *Mol. Gen. Genetics* **207**, 421 (1987); S. E. Adams *et al.*, *Cell* **49**, 111 (1987).
25. D. J. Garfinkel, A. Hedge, S. D. Youngren, T. D. Copeland, *J. Virol.* **65**, 4573 (1991).

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